RAPID OPTIMIZATION OF CHROMATOGRAPHY OPERATING CONDITIONS USING A NANO-LITER SCALE COLUMN ON A MICROFLUIDIC CHIP WITH INTEGRATED PNEUMATIC VALVES AND OPTICAL SENSORS

Inês F. Pinto, iBB – Institute for Bioengineering and Biosciences, Instituto Superior Técnico and INESC-Microsystems and Nanotechnologies, Lisbon, Portugal
ines.f.pinto@tecnico.ulisboa.pt
Denis R. Santos, INESC-MN and iBB
Ruben R.G. Soares, INESC-MN and iBB
Virginia Chu, INESC-MN
M. Raquel Aires-Barros, iBB, Instituto Superior Técnico
João P. Conde, INESC-MN, Instituto Superior Técnico
Ana M. Azevedo, iBB, Instituto Superior Técnico

Key Words: Chromatography, Monoclonal Antibodies, Microfluidics, Screening

Purification of monoclonal antibodies (mAbs) is traditionally achieved by chromatographic separations, which are very robust but require time-consuming optimization on a case-by-case, particularly if a non-affinity step is used. In this context, multimodal chromatography has been explored as a versatile and cost-effective alternative to the established affinity step employed for capturing mAbs. However, selective capture/polishing of a target mAb using such multimodal ligands comes with the need for extensive and time-consuming optimization, due to the multitude of interactions that can be simultaneously promoted in the ligand. In this work, we developed a novel microfluidic platform comprising multimodal chromatography beads inside micro-columns for rapid screening of operating conditions. Sequential liquid insertion in the device was achieved by using integrated pneumatic valves and the chromatographic assays were combined with a signal acquisition module for on-chip fluorescence measurements.

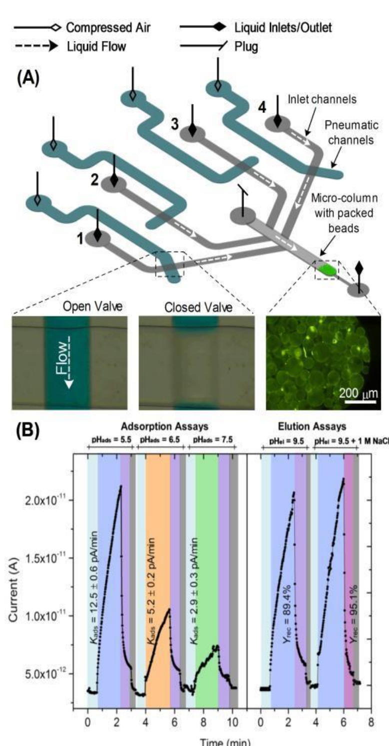

Figure 1 – (A) Schematics of the microfluidic structure used for chromatography screening assays. (B) Binding curves for the mAb-ligand interaction at different conditions.

The chromatographic multimodal ligand Capto MMC was studied for the capture and elution of a fluorescent conjugate (mAb-Alexa 430), spiked in a real cell culture supernatant, under different pH and conductivity conditions. Micro-columns (V = 210 nL) were fabricated in polydimethylsiloxane (PDMS) for packing the agarose beads and the screening studies were performed by flowing 4 different solutions through the column in sequence (Figure 1-A). Binding kinetics were measured in real-time at bead-level by fluorescence microscopy and by aligning the micro-columns with 200 um *a*-Si:H photodiodes (Figure 1-B). Chromatographic cycles comprising (1) equilibration, (2) adsorption, (3) elution and (4) regeneration of the packed beads were performed in series using the same device without detriment to the results. Regarding the signal acquisition using photosensors, the fluorescence signal of the beads was continuously monitored by illuminating the column using a 405 nm blue-violet laser while measuring the current generated by a photodiode at 0 V bias during the different stages of the chromatography cycle. Apart from studying the interaction of Capto MMC with the target mAb, Carboxymethyl Sepharose and Phenyl Sepharose resins were additionally selected to evaluate the individual effect of electrostatic and hydrophobic interactions, respectively, in governing the binding on the multimodal ligand.

In summary, we report an integrated microfluidic-based approach to effectively perform early-stage optimization of chromatographic separations, with very low molecule (~2.5 ug mAb), reagent (<50 uL buffer) and resin (~70 nL) consumption and extremely rapid output of results (<2 min/condition tested *vs* 20/30 min in a conventional chromatographic run).

References:
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